

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

**Unexpected *Listeria monocytogenes* detection with a dithiothreitol-based device during an aseptic hip revision [\*G. Banche and A. Bistolfi contributed equally to this work; \*\* V. Allizond is the corresponding author]**

**This is a pre print version of the following article:**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1678111> since 2020-08-31T14:53:52Z

*Published version:*

DOI:10.2217/fmb-2018-0135

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

## Title Page Template

- **Article title:** Unexpected *Listeria monocytogenes* detection with a dithiothreitol based device during an aseptic hip revision
- **Short running title:** *L. monocytogenes* infection detected by DTT
- **Author names:** Giuliana Banche<sup>1\*</sup>, Alessandro Bistolfi<sup>2\*</sup>, Valeria Allizond<sup>1</sup>, Claudia Galletta<sup>2</sup>, Maria Rita Iannantuoni<sup>1</sup>, Elisa Simona Marra<sup>1</sup>, Chiara Merlino<sup>1</sup>, Alessandro Massè<sup>2</sup>, Anna Maria Cuffini<sup>1</sup>  
\*Both authors contributed equally to this work
- **Author affiliations:**  
<sup>1</sup>Department of Public Health and Pediatrics, University of Torino, via Santena 9, 10126 Turin, Italy  
<sup>2</sup>Department of Orthopedics and Traumatology, AOU Città della Scienza e della Salute, CTO Hospital, Via Zuretti 29, 10126 Turin, Italy
- **Corresponding author details:**  
Dr. Valeria Allizond  
Microbiology Section, Department of Public Health and Pediatrics,  
University of Torino,  
Via Santena 9, 10126 Turin, Italy  
Phone: +390116705644 Fax: +390112365644  
Email address: valeria.allizond@unito.it
- **Financial disclosure:** all the authors disclose any financial and material support for the present research. All the authors also disclose any personal, academic or financial relationships that could influence their actions relationships and financial involvement with an organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.
- **Acknowledgements:**  
The authors thank Fondazione CRT for sponsoring this study.
- **Information pertaining to writing assistance:** N/A
- **Ethical disclosure:** N/A
- **Author Contributions:** Giuliana Banche and Alessandro Bistolfi masterminded the paper, dealt with data interpretation and wrote the paper; Valeria Allizond cooperated in the design of the study, dealt with the acquisition of the data and critically revised the paper; Maria Rita Iannantuoni and Elisa Simona Marra processed the samples and analyzed the microbiological data; Claudia Galletta cooperated in the design of the study and analyzed the clinical data; Chiara Merlino and Alessandro Massè critically revised the paper; Anna Maria Cuffini cooperated in the design of the study, dealt with data interpretation and critically revised the paper.

**Word count:** 1.646

**Figure number:** 2

**Table number:** 0

## Article Body Template

- **Abstract:**

Prosthetic joint infection (PJI) diagnosis is often difficult since biofilm-embedded microorganisms attach well to the prosthetic surfaces and resist their detection by conventional methods. DL-dithiothreitol (DTT) has been described as a valid method for biofilm detachment on orthopedic devices. We report the case of an occasional detection of *Listeria monocytogenes* in a non-immunocompromised patient with a pre-operative diagnosis of aseptic loosening. The infection diagnosis due to such rare bacteria was made post operatively thanks to a DTT based device: it maybe considered a feasible approach for the microbiological analysis of PJIs considering that a prompt diagnosis of such biofilm-associated infections could bring some advantages such as an early and appropriate antibiotic therapy administration and a reduction of undiagnosed infections.

- **Keywords:**

Prosthetic joint infections, DL-dithiothreitol, *Listeria monocytogenes*

- **Main body of text:**

The number of prosthetic joint infections (PJIs) is increasing worldwide. The literature data indicate that patients with PJIs have a significantly higher number of re-admissions, longer hospital stays with very high costs that exceed those of patients who have had non-infected prosthetic implant [1]. Therefore it follows that the detection of a pathogen is essential for the choice of a suitable antibiotic therapy to treat PJIs and hence eliminate the severity of sequelae. The suspect of infection must be taken into account for any painful implant, even in absence of clinical signs: PJIs may be subclinical, without major signs of infections such as fistula and redness and pre-operative tests, scintigraphy and markers [(C-reactive protein (CRP) and erythrocyte sedimentation rate] may not be significant. In addition, intra-operative cultures during revision surgery are often negative due to antibiotic therapy and limits of diagnostic methods (intra-operative swabs and cultures). Therefore, during revision joint surgery, a most accurate method for establishing the presence or absence of a microbial infection is necessary [2,3].

The main problem of microbiological diagnosis of infection is that biofilm-embedded microorganisms are well attached to prosthetic surfaces and to each other and are not detected by conventional methods, hence leading to negative results of cultural analysis of swabs and aspirates. A high number of bacteria responsible for PJIs is capable of producing biofilm matrix on prosthetic implants thanks to their affinity for foreign bodies [4]. Bacterial adhesion and following biofilm formation depend on many factors, including the bacterial type and its load, the physical and chemical environmental conditions, the presence of nutrient factors, the hydrodynamic forces and the substrate nature [5-7].

Several procedures able to detach bacteria from biofilm on prosthetic implants have been applied to increase microbial recovery with the aim of making better microbiological assay sensitivity [2]. Sonication, a technique for PJI diagnosis, based on the long-wave ultrasound action, has been described by Trampuz et al. in 2007 [8]. The method removes bacteria from the implant surfaces and the subsequent quantification of the microorganism number may help to distinguish infected from contaminated prostheses [3,8]. However, this procedure maintains some drawbacks such as the handling of large implants with the risk of cross-contamination [4].

In recent times, the application of chemical substances to remove bacteria from biofilm matrix has been described as a valid resolution to overcome these disadvantages preserving good level of sensitivity and specificity. DL-dithiothreitol (DTT) is a sulfhydryl compound able to reduce disulfide bonds so that the detachment of biofilm from orthopedic prosthesis is possible [2].

---

## Article Body Template

In this paper, we report the case of joint infection sustained by *Listeria monocytogenes* in an immunocompetent patient with a suspected aseptic loosening, diagnosed by a DTT based device. *L. monocytogenes*, a foodborne pathogen able to form biofilm under various conditions, is an unusual cause of bone and joint infections that usually occur in immunocompromised patients such as those affected by cancer, diabetes, renal or liver dysfunction [9-12].

### Case description

We describe the clinical case of a 78 years old non-immunocompromised woman with bilateral total hip arthroplasty (THA, right implanted in 1995 and left in 1996). The patient did not suffer for any relevant anamnestic clinical condition, except for a rectal cancer surgically treated in 1983. She did not have others comorbidities and she did not take corticosteroids or chemotherapy. In 2003, the right stem was revised for aseptic loosening, with good recovery and without complications. In 2013 the patient underwent right total knee arthroplasty for osteoarthritis. In July 2017 she was admitted at the Department of Orthopedics and Traumatology, AOU Città della Scienza e della Salute, CTO Hospital (Turin, Italy) for severe left hip pain since approximately six months with inability to weight bearing and painful restriction of left hip motion. She had leukocytosis without fever. The CRP was 24 mg/dL. The scintigraphy was negative. Plan x-radiographs showed signs of femoral stem mobilization (Figure 1). Therefore, the patient was scheduled for a one-stage revision of the stem of the left THA, with the diagnosis of aseptic loosening. The patient received vancocine 1 gr ev 1 hour pre-operatively and a second dose of vancocine 2 gr after 8 hours. The intra-operative swabs, subjected to routine microbiological cultural analysis, gave no evidence of microbial growth. Post-operative recovery was good in terms of pain, motion, wound healing and was uneventful in terms of infection. Figure 2 shows the routine x-ray at six months.

### Implant microbiological analysis

The removed prosthesis stem and peri-implant tissues were placed in sterile and safe conditions into the microDTTect device (4i s.r.l., Monza, MB, Italy), a device for collection, transport and treatment of samples based on DTT, and sent to Bacteriology and Mycology Laboratory of the Department of Public Health and Pediatrics, University of Torino (Turin). The device was placed on a mechanical shaker for 15 minutes to detach biofilm and bacteria from tissues and biomaterial surfaces. The DTT solution was transferred into dedicate test tubes that were centrifuged at 3000 rpm for 10 minutes. The obtained pellet was used to perform microbiological cultures. In details, 100 µl were plated on Columbia + 5% sheep blood agar (BA) and Chocolate agar (bioMérieux Italia Spa, Bagno a Ripoli, FI, Italy) for aerobic bacteria, Schaedler + 5% sheep blood agar (bioMérieux) for anaerobic bacteria, Sabouraud Dextrose agar (Merck KGaA, Darmstadt, Germania) for fungi; and 100 µl were placed in Brain Heart Infusion (BHI) broth (Merck KGaA) for aerobic bacteria, Thioglycollate broth (Merck KGaA) for anaerobic bacteria and Sabouraud dextrose broth (Merck KGaA) for fungi.

All the cultural media were incubated at 37°C in aerobic or anaerobic conditions and monitored for at least 14 days before to be discharged as negative. A growth of  $\geq 50$  colony forming units/ml on agar, or turbidity in broth, was considered positive.

The BA plate and the BHI broth revealed the presence of Gram-positive and catalase-negative bacilli, identified as *L. monocytogenes* using the API Listeria system (bioMérieux).

### Conclusions

The biofilm mode of growth is highly relevant in clinical settings where these communities cause several chronic infections and pose diagnostic problems. A fast diagnosis of biofilm related infection is important for the successful

## Article Body Template

of the antibiotic therapy, hence it is fundamental to distinguish implant septic loosening from aseptic one (3). It is possible that a certain part of the aseptic loosening revisions is a subclinical septic loosening. Although probably part of these infections is reduced during the revision surgery thanks to several factors such as the antibiotic therapy, the surgical wash and the removal of the peri-prosthetic bone, a certain diagnosis about the presence or absence of a microorganism is still mandatory. In case of bacterial detection, first of all a specific antibiotic therapy can be given to the patient and, last but not least, in case of secondary infection development, this would be not related to the revision surgery but classified as pre-existing with huge medico-legal importance.

Consequently, the need to find a more accurate and sensitive method for bacterial isolation emerges. In our case, the DTT based device, that is not commonly used in the clinical practice, made it possible to find biofilm producing bacteria such as *L. monocytogenes*. This bacterium is an unusual cause of bone and joint infections, which usually occur in immunocompromised patients [9-12]; nevertheless, *L. monocytogenes* occasionally can be agent of PJIs, even in immunocompetent subjects [9-10]. The use of DTT, as a valid practice in place of sonication, thanks to its higher positive and negative predictive values and rapidity to process samples [4], has been finally approved and published in the Italian Association of Clinical Microbiologists (AMCLI) guidelines (<http://www.amcli.it/documenti/percorsi/percorsi-2013/>). Furthermore, the microDTTect device, easy to use, is a completely closed method that avoids cross-contaminations: it preserves samples from any contamination in every single step (harvesting, transporting, processing and culturing) to prevent false positive cultures and detect bacteria responsible for surgical site infections.

In this clinical case, the favorable patient outcome depended also on the characteristics of *L. monocytogenes* that fortunately was vancomycin susceptible, so the signs of infection were not evident before the surgical revision. In any case, after detecting *L. monocytogenes*, we strictly monitored the patient to be ready to start a different antibiotic therapy if necessary.

Lastly, this clinical case suggests that PJIs may be caused even by rare and unusual microorganisms that may not be detected with conventional methods. Cost analysis does not fall within the aim of our work, but it is possible to assume that DTT based device costs would be largely covered by the money saved both from infections, and thus, from surgical new revisions, and from specific antibiotic therapy that would no longer be necessary to administer until evident infection.

The clinical impact of this case is related to the following points: 1) any painful arthroplasty with no clear reason should be considered infected; 2) revision surgery can solve subclinical infections although not recognized; 3) specific microbiological analysis must be used in addition to routine swab cultures; 4) accurate diagnosis of infection may reduce complication rates and second revisions.

### • Summary Points:

- ✓ The diagnosis of prosthetic joint infections (PJIs) is often difficult.
- ✓ The main problem for microbiological PJI diagnosis is that biofilm-embedded microorganisms attach and resist well to the prosthetic surfaces.
- ✓ DL-dithiothreitol (DTT) has been described as a valid method for the detachment of biofilm growing on orthopedic devices.
- ✓ An immunocompetent 78 years old female with bilateral total hip arthroplasty presented severe left hip discomfort, incapacity to weight bearing, painful restriction of left hip motion.
- ✓ The explanted stem was studied with a DTT based device
- ✓ The microbiological cultural analysis of DTT solution revealed the presence of *L. monocytogenes*.

## Article Body Template

- ✓ DTT device maybe considered a feasible approach for the microbiological analysis of PJs considering that a prompt diagnosis of such biofilm-associated infections could bring some advantages.

- **Figure legends:**

Figure 1. Plan x-radiographs showed signs of femoral stem mobilization

Figure 2. Routine x-ray at six months

- **References:**

- [1] Calori GM, Colombo M, Navone P, *et al.* Comparative evaluation of MicroDTTect device and flocked swabs in the diagnosis of prosthetic and orthopaedic infections. *Injury*. 47 Suppl 4, S17-S21 (2016). **\*\* This article underlines the importance of the competent use of the microDTTect devices as innovative technology to dislodge bacterial biofilm from prosthetic surfaces of explanted samples.**
- [2] De Vecchi E, Bortolin M, Signori V, Romanò CL, Drago L. Treatment with dithiothreitol improves bacterial recovery from tissue samples in osteoarticular and joint infections. *J. Arthroplasty*. 31(12), 2867-2870 (2016). **\*\* In this article the importance of the dithiothreitol to recover bacteria embebbled in biofilm on orthopedic samples is proved.**
- [3] Tani S, Lepetsos P, Stylianakis A, Vlamis J, Birbas K, Kaklamanos I. Superiority of the sonication method against conventional periprosthetic tissue cultures for diagnosis of prosthetic joint infections. *Eur. J. Orthop. Surg. Traumatol.* 28(1), 51-57 (2018).
- [4] Drago L, Signori V, De Vecchi E, *et al.* Use of dithiothreitol to improve the diagnosis of prosthetic joint infections. *J. Orthop. Res.* 31(11), 1694-1699 (2013).
- [5] Banche G, Bracco P, Bistolfi A, *et al.* Vitamin E blended UHMWPE may have the potential to reduce bacterial adhesive ability. *J Orthop Res.* 2011;29(11):1662-1667.
- [6] Banche G, Allizond V, Bracco P, *et al.* Interplay between surface properties of standard, vitamin E blended and oxidised ultra high molecular weight polyethylene used in total joint replacement and adhesion of *Staphylococcus aureus* and *Escherichia coli*. *Bone Joint J.* 96-B(4), 497-501 (2014). **\*\* This research article demonstrated the importance to study new biomaterials for implants to avoid periprosthetic infections.**
- [7] Banche G, Bracco P, Allizond V, *et al.* Do crosslinking and vitamin E stabilization influence microbial adhesions on UHMWPE-based biomaterials? *Clin. Orthop. Relat. Res.* 473(3), 974-986 (2015).
- [8] Trampuz A, Piper KE, Jacobson MJ, *et al.* Sonication of removed hip and knee prostheses for diagnosis of infection. *N. Engl. J. Med.* 357(7), 654-663 (2007). **\* This article represents the milestone into the microbiological analysis of retrieved prostheses by using sonication.**
- [9] Chougale A, Narayanaswamy V. Delayed presentation of prosthetic joint infection due to *Listeria monocytogenes*. *Int. J. Clin. Pract.* 58(4), 420-421 (2004).
- [10] Mavrogenis AF, Savvidou OD, Vlasik K, Papagelopoulos PJ. Late hip arthroplasty infection caused by *Listeria monocytogenes* in a non-immunocompromised patient. *Surg. Infect. (Larchmt)*. 12(2), 137-140 (2011). **\* This article reports a case comparable to our: a prosthetic joint infection in an immunocompetent subject sustained by *Listeria monocytogenes*.**
- [11] Charlier C, Leclercq A, Cazenave B, *et al.* *Listeria monocytogenes*-associated joint and bone infections: a study of 43 consecutive cases. *Clin. Infect. Dis.* 54(2), 240-248 (2012).

---

### Article Body Template

- [12] Bader G, Al-Tarawneh M, Myers J. Review of prosthetic joint infection from *Listeria monocytogenes*. *Surg. Infect. (Larchmt)*. 17(6), 739-744 (2016). \*\* In this review cases of association between prosthetic joint infection and *Listeria monocytogenes* are presented.

R





